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6. AUTHOR(S)
MARTIN L. MELTZ, PH.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO
DEPARTMENT OF RADIATION ONCOLOGY
7703 FLOYD CURL DRIVE
SAN ANTONIO, TX 78229-3900

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13. ABSTRACT *(Maximum 200 words)*

The most significant finding occurred after exposure of huma 244B lymphoblastoid cells to a high-peak power pulsed ultrawideband transmitted electric field (UWB TEMF) signal, which had gived a wide array of largely negative results. The UWB TEMF exposure (average peak power, 100 kV/m; average pulse width, 780 ps) duration was 90 min (intermittent). The UWB TEMF did not cause an alteration in cell cycle distribution, stabilization of the p53 target genes. There was no loss of mitochondrial membrane potential or release of cytochrome C into the cytosol at 6 hr post-exposure (i.e., no apoptosos). However, at 2 hr a number of gene increase and decrease were detected (12,000 gene microarray system). This suggests that the cells were capable of "sensing" the pulsed UWB TEMF. While the induction of the gene transcription factor NF-kB was observed, no evidence was obtained for downstream activity. The UWB TEMF signal may be necessary, but noit sufficient to cause coordinated downstream events. FDTD analysis was performed to determine the doses for exposure cells in suspension or surface attached. An unexpected distribution of energy in the medium is described.

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Cellular, Molecular Signaling and Genetic Alterations After High-Peak Power Pulsed and Ultrawideband Radio Frequency Radiation Exposure

Abstract

The most significant finding occurred after exposure of human 244B lymphoblastoid cells to a high-peak power pulsed ultrawideband transmitted electric field (UWB TEMF) signal, which had given a wide array of largely negative results. The UWB TEMF exposure (average peak power, 100 kV/m; average pulse width, 780 ps) duration was 90 min (intermittent). The UWB TEMF did not cause an alteration in cell cycle distribution, stabilization of the p53 gene or transactivation of p53 target genes. There was no loss of mitochondrial membrane potential or release of cytochrome C into the cytosol at 6 hr post-exposure (i.e., no apoptosis). However, at 2 hr a number of gene increases and decreases were detected (12,000 gene microarray system). This suggests that the cells were capable of "sensing" the pulsed UWB TEMF. While the induction of the gene transcription factor NF-*k*B was observed, no evidence was obtained for downstream activity. The UWB TEMF signal may be necessary, but not sufficient to cause coordinated downstream events. FDTD analysis was performed to determine the doses for exposure of cells in suspension or surface attached. An unexpected distribution of energy in the medium is described.

1. **Period covered:**
15 May, 2001 to 31 August, 2004 (Final Report)
Including Annual Report for the period 1 September 2003 to 31 August 2004

2. **Title of Proposal:**

Cellular, Molecular Signaling and Genetic Alterations After High-Peak Power Pulsed and Ultrawideband Radio Frequency Radiation Exposure

3. **Grant Number:** F49620-01-1-0349

4. **Name of Institution**:

University of Texas Health Science Center at San Antonio

5. **Author(s) of Report:**

Martin L. Meltz, Ph.D., Principal Investigator (210-567-8025;
meltz@uthscsa.edu)
Bijaya Nayak, Ph.D., Co-Investigator (210-567-8033; nayak@uthscsa.edu)
Mohan Natarajan, Ph.D., Co-Investigator (210-567-5654; natarajan@uthscsa.edu)
Fax Number for listed Investigators: 210-567-8051

Address: Department of Radiation Oncology
Univ. of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78229-3900

Satnam Mathur, Collaborating Senior Electrical Engineer, Formerly
McKesson Bioservices
U.S. Army Medical Research Detachment
Brooks AFB, TX 78235

6. **Manuscripts submitted/published:**

RF Papers Published:

Natarajan M, Roldan FN, Vijayalaxmi, Szillagyi M, Meltz ML. NF-*kB* DNA-binding activity after High Peak Power Pulsed Microwave (8.2 GHz) Exposure of Normal Human Monocytes.. Bioelectromagnetics 23: 271 – 277 (2002).

Ziskin MC. Meltz ML, et. al. (25 co-authors) Medical Aspects of Radiofrequency Radiation Overexposure Health Physics 82(3): 387-391 (2002)

Meltz, ML: Radiofrequency Exposure and Mammalian Cell Toxicity, Genotoxicity, and Transformation. Bioelectromagnetics, Supplement 6, S196-S213 (2003)

RF and UWB TEMF Papers submitted:

Nuclear translocation and DNA-binding Activity of NF-*k*B upon ultra-wideband electromagnetic radiation exposure fails to transactivate *k*B-dependent gene expression in human monocytes. M Natarajan, BK Nayak, SL Pandswara, FN Roland, C Galindo, ML Meltz, SP Mathur (Submitted to RRS, 2004)

Effect of ultrawideband electromagnetic fields on cell cycle progression in human leukemic HL-60 cells. BK Nayak, NM Natarajan, C Galindo, SP Mathur, and ML Meltz (Submitted to Bioelectromagnetics, 2004)

Determination of p53 protein stabilization and transactivation of its target genes in response to UWB electromagnetic field exposures in hematopoietic cells. BK Nayak, M Natarajan, C Galindo, S Mathur, and ML Meltz (In Revision, Radiation Research, 2004)

RF and UWB TEMF Presentations at meetings over the course of the Project:

Z. Ji, S. C. Hagness, J. H. Booske, S. Mathur, and M. MELTZ, FDTD Analysis of a Gigahertz TEM Cell for Ultrawideband Pulse Exposure Studies of Biological Specimens, IEEE Antennas and Propagation Society International Symposium and USNC/URSI Radio Science Meeting, Monterey, CA, June 2004.

M Natarajan, BK Nayak, SP Mathur, C Galindo, and ML MELTZ. Ultrawideband electromagnetic radiation (UWB EMR) exposures and activation of the signal transduction pathway. Presented at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 20-24, 2004

BK Nayak, C Galindo, M Natarajan, SP Mathur, and ML MELTZ. Determination of P53 protein stabilization, loss of mitochondrial membrane potential, and the release of cytochrome C into the cytosol in response to UWB EMR exposure in human lymphoblastoid cells." Presented at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 20-24, 2004

ML MELTZ, BK Nayak, C Galindo, and M Natarajan. Nanosecond UWB EMR EMF pulses effect cell recovery and viability, and result in the induction of c-fos oncogene expression in human lymphoblastoid cells." Presented at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 20-24, 2004

Z Ji, SC Hagness, JH Booske, S Mathur, and M MELTZ. Finite-difference time-domain (FDTD) analysis and dosimetry of a gigahertz TEM cell. [This paper was a collaboration with investigators in the Dept. of Electrical and Computer Engineering, Univ. of Wisconsin, Madison] Presented at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 20-24, 2004

M MELTZ, C Galindo, B Nayak, M Natarajan, N Vela-Roch, and S Weintraub. Human cell recovery, viability, cell cycle progression and proliferation over 2-72 hours post 10 ns extremely high peak power pulsed UWB EMF exposures. Presented at the MURI Symposium at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 24, 2004

M Natarajan, FA Roldan, B Nayak, C Galindo, N Vela-Roch, S Weintraub, and M MELTZ. Nanosecond UWB-EMF pulses differentially modulate transcriptional regulators in Jurkat T-lymphoma cells. Presented at the MURI Symposium at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 24, 2004

B Nayak, C Galindo, S Weintraub, N Vela-Roche, M Natarajan, and M MELTZ. Alterations in apoptotic and anti-apoptotic genes and Fos/Jun families of transcriptional factors in response to pulsed 10 ns ultrawideband electromagnetic field exposures. Presented at the MURI Symposium at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 24, 2004

S Weintraub, N Vela-Roch, C Galindo, M Natarajan, B Nayak, and M MELTZ. Parameter optimization for differential protein expression (proteomic) analysis of 10 ns UWB-EMF exposed human cells. Presented at the MURI Symposium at the 26th Annual Meeting of the Bioelectromagnetics Society, Washington D.C., June 24, 2004

MELTZ ML, Nayak BK, Natarajan M, Galindo C, Mathur SP. Transcription of p53 target genes in response to ultrawideband electromagnetic radiation exposure in human cells. 25th Annual Meeting, Bioelectromagnetics Society, Maui, Hawaii, June 22-27, 2003.

MELTZ ML, Nayak BK, Galindo C, Mathur SP, and Natarajan M. Effects of ultrawideband electromagnetic radiation on cell cycle progression in human cells. 25th Annual Meeting, Bioelectromagnetics Society, Maui, Hawaii, June 22-27, 2003.

MELTZ ML, Natarajan M, Nayak BK, Roldan FA, Galindo C, and Mathur SP,. Genomic profiling of NFkB signal-dependent genes in human monocytes after ultrawideband electromagnetic radiation exposure. 25th Annual Meeting, Bioelectromagnetics Society, Maui, Hawaii, June 22-27, 2003.

Martin L. MELTZ, Radiological Terrorism Preparedness-Current Activity of the South Texas Chapter's Nuclear Training Endeavors Task Force. Health Physics Society's 36th Midyear Meeting, San Antonio, Texas. January 26-29, 2003.

Martin L. MELTZ, In Vitro Investigations of Potential Interactions of Radio frequency Radiation with other Physical and Chemical Agents. Twenty-fourth Annual Bioelectromagnetics Society Meeting, Quebec, Canada. June 23-27, 2002.

Martin L. MELTZ, Cynthia Galindo, Mohan Natarajan, Robin Leach, Xavier Reveles, Satnam Mathur and John Ashmore. Investigation of the ability of UWB RF exposure to induce translocations in human chromosomes 1, 2 or 4 using multicolor FISH technology. Ninth Annual Michaelson Research Conference, Portland, Maine, August 9 – 12, 2002.

Mohan Natarajan, Martin L. MELTZ, Cynthia Galindo, John Ashmore, and Satnam Mathur. Influence of UWB RF Exposure on Nuclear Translocation of NF-*k*B in Human MM-6 Monocytes. Ninth Annual Michaelson Research Conference, Portland, Maine, August 9-12, 2002.

Invited Presentations

Invited presentation at a special meeting on the "Influence of RF Fields on the Expression of Heat-Shock Proteins". Sponsored by Forschungsgemeinschaff Funk e.V (FGF) Research Association for Radio Applications, in cooperation with the World Health Organization (WHO), the European Research Action COST 281, and the STUK Finland (Radiation and Nuclear Safety Authority). Helsinki, Finland, June 27-29, 2004. Dr. Meltz was asked to give an overview talk entitled "Review of RF-effects on HSPs in the Context of Biological and Health Effects." At the meeting, he was also asked to lead the Summary Discussion in the final session.

Invited presentations in the Asia-Pacific EMF Conference in Bangkok, Thailand, January 26-30, 2004. The meeting was co-sponsored by the World Health Organization (WHO), the Thailand Ministry of Public Health, the United States Air Force Research Laboratory, Health Canada, and the Association of Thai Professionals in America and Canada. The title of his first talk, in the tutorial session, was "A Report on an RF-Induced Biological Effects Versus an Adverse Human Effect: Science Versus Speculation." The title of his second talk, in the technical session, was Mammalian Cell Toxicity, Genotoxicity, and Transformation after Radiofrequency Exposure.

Invited presentation for 3rd Intl EMF Seminar in China-Electromagnetic Fields and Biological Effects, Critical Evaluation of In Vitro and related In Vivo reports of Radio Frequency Radiation Exposures, Guilin, China, October 13-17, 2003.

8. Inventions/Patents/Discoveries

None

9. Collaborators/Consultants:

Satnam Mathur, Senior Electrical Engineer, McKesson BioServices, Contractor for the U.S. Army Medical Research Detachment, Brooks AFB, TX.

Satnam Mathur has assisted our Research Group in obtaining dosimetry measurements for exposure of suspended mammalian cells in T-25 flasks to UWB RF in the GTEMS

unit he operates for the U.S. Army. He oversees the operation of the unit, as a collaborator, in the performance of the UWB exposures to be undertaken in this project. He has trained Dr. Meltz to operate the unit, and Cynthia Galindo to operate the temperature monitoring and control system.

Dr. Johnathan Kiel, Chief, Mechanisms Branch, AFRL, Brooks AFB, is a collaborator.

Dr. Kiel and the members of his research group, and the members of our research group, are examining unique RF exposures at different biological levels. The UTHSCSA Radiobiology Group focuses on *in vitro* exposures of mammalian cells.

Dr. Susan C. Hagness, Dept. of Electrical and Computational Engineering
Univ. of Wisconsin-Madison, Madison, WI

Dr. Hagness is an expert in the area of FDTD analysis. She has performed an FDTD analysis, under a subcontract to the University of Wisconsin – Madison, to determine the SAR pattern in T-25 flasks with cells attached to the flask surface closest to the source of an TUWB EMF signal. Until this was done, because of the limitations of UWB dosimetry, we had been limited our research activity to exposing cells grown in suspension. The completed work is described herein.

Stewart Allen, RF Engineer, General Dynamics Corporation, Contractor for the U.S AFRL, Brooks City-Base, Texas

Mr. Allen has extensive expertise in RF dosimetry equipment, exposures set-ups, and dosimetry. He has provided extensive information about the temperature uniformity and SAR profiles (described in this report) for the 2.8 GHz narrowband exposure system.

10. Honors or Awards received by you or your personnel while being supported by AFOSR over the past year.

None Received

11. Key Findings/Results/Accomplishments:

PROJECT TITLE:

Effects of UWB Transmitted Electromagnetic Fields (TEMF) on Cell Cycle Progression of Mammalian Cells
Lead Investigator: Bijaya K Nayak, PhD
Associated Investigators: Cynthia Galindo, Satnam P Mathur, Martin Meltz

For the summary of research in this area:
See the attached draft manuscript with the file name:

UWB TEMF cell cycle paper-revised June, 2004.doc

PROJECT TITLE:

Effects of UWB TEMF on p53 and transcription of its target genes
Lead Investigator: Bijaya K Nayak, PhD
Associated Investigators: Cynthia Galindo, Satnam P Mathur

Manuscript in Revision, Sept. 2004
See Attached Manuscript with Adobe Acrobat PDF File Name:

UWB TEMF Effect on Transcription of p53 and its Target Genes-In Rev 2004.pdf

PROJECT TITLE:

Effects of UWB TEMF on RNA transcription in mammalian cells assessed using microarray (genomics) discovery methodology (12,000 human gene array)

Lead Investigator: Martin Meltz, PhD
Associated Investigators: Bijaya Nayak, Cynthia Galindo, Satnam Mathur

Objective:

The aim of the study was to compare the genomic profile of 244B cells exposed to UWB EMR to mock exposed cells in order to determine if UWB exposure causes up-regulation or down-regulation of any of 12,000 human genes.

Methodology:

The studies were performed in 244B human lymphoblastoid cells. The cells were exposed to UWB EMR pulses intermittently for a total of 90 minutes (30 min on, 30 min off). The UWB EMR pulses had an average peak amplitude of 100 kV/m, an average pulse width of 0.80 ns, an average rise time of 200 ps, and a pulse repetition frequency of 250 pps. The frequencies ranged from D.C. to ~2 GHz. 12K human plastic microarrays from Clontech were used.

RNA isolation was performed using TriZol following manufacturer's directions. The RNA pellet was treated with DNase. The RNA yield was determined by measuring A_{260} (1 A_{260} unit of RNA = 40 μg/ml). The purity was calculated taking the A_{260}/A_{280} ratio. Pure RNA exhibits a ratio of 1.9-2.1. The quality of RNA was checked by electrophoresis in 1% denaturing agarose gel. Poly A+ RNA enrichment was performed using magnetic streptavidin beads and following manufacturer's instructions. cDNA probe was synthesized and the labeled cDNA from unincorporated ^{33}P-labelled nucleotides and small (<0.1 Kb) cDNA fragments was purified by column chromatography. cDNA probes were hybridized to the plastic array overnight with continuous rocking at 60 deg C. The membranes were then washed and exposed to phosphorimager screen suitable for ^{33}P detection for 24 hours. The phosphorimager screen was scanned at a resolution of 50μm.

Analysis was performed using AtlasImage software v2.7 to compare treated to untreated arrays.

The arrays were first aligned to the Grid Template in order to allow the software to determine the location of all the genes on the array. After producing an overall alignment that approximately matches the Grid Template to most of the genes, the alignment was fine tuned in several different ways to ensure that there were no splotchy or uneven areas in the array.

The aligned arrays were then normalized using global (default) method and user defined housekeeping genes actin and GADPH. Ratio Threshold Value was set to 2 and the Difference Threshold Value was set to 100. A report was generated of all the genes which have met the defined criteria for inclusion onto the list as up-regulated or down-regulated.

The data analysis was put into spreadsheet tabular format.

Results:

For the 2 hr post exposure incubation, several genes were found to be different in treated vs. sham exposed samples.
The settings for comparison of two aligned arrays were 2.0 for fold ratio and 100 unit difference in adjusted intensity. Only those genes which had both 2 fold ratio and 100 unit intensity difference were included in the report. We found 73 genes were up-

regulated and 30 genes were down-regulated using both of the user-defined conditions. Of interest, several oncogenes were up-regulated: ret finger protein, r-ras (related RAS viral oncogene), v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, met proto-oncogene (hepatocyte growth factor receptor), v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblator, glioma amplified on chromosome 1 protein (leucine-rich). DNA repair associated genes were up-regulated as well: DNA topoisomerase II alpha and Replication protein A1. Also of interest, some transcription factors were down-regulated: CREB/ATF family transcription factors and p300/CBP associated factor.

For the 24 hr post exposure incubation, several genes were found to be different in treated vs. sham exposed samples.
The settings for comparison of the two aligned arrays were 5.0 fold ratio with 0 unit intensity difference and 3.0 fold ratio with 0 unit intensity difference. The 5 fold ratio showed between 5 and 10 genes being up-regulated or down-regulated depending on which of three different normalization methods were used. However, three genes were common to each of the normalized methods. Those genes are RAB3A (member of RAS oncogene family), perilipin, and immunoglobulin lambda joining 3. Using the same three normalization methods and ratio set to 3.0 fold, there were between 28 and 40 genes up-regulated. The three normalization methods had 22 genes in common as being up-regulated or down-regulated.

UWB EMR Exposure, 24 hr time point
Analysis Report 1
No Normalization, Ratio=5, Difference=0

Ratio	Protein/gene	Accession
6.43	major histocompatibility complex, class I, A	NM_002116
12.39	RAB3A, member RAS oncogene family	NM_002866
5.64	ribosomal protein L19	NM_000981
6.55	arylsulfatase A	NM_000487
5.61	ribosomal protein L13a	X56932
5.95	protein tyrosine phosphatase, non-receptor type 9	NM_002833
8.56	perilipin	NM_002666
5.17	KIAA0907 protein	NM_014949
5.35	pleiotropic regulator 1 (PRL1homolog, Arabidopsis)	NM_002669
10.57	immunoglobulin lambda joining 3	NM_016934

Analysis Report 2
USER DEFINED NORMALIZATION, ACTIN (3 GENES) AND GAPDH (3 GENES)

Ratio	Protein/gene	Accession
5.42	major histocompatibility complex, class I, A	NM_002116
10.44	RAB3A, member RAS oncogene family	NM_002866
5.51	arylsulfatase A	NM_000487
7.22	perilipin	NM_002666
8.91	immunoglobulin lambda joining 3	NM_016934

Analysis Report 3
GLOBAL NORMALIZATION (SUM METHOD), Ratio=5, Difference=0

Ratio	Protein/gene	Accession
9.21	RAB3A, member RAS oncogene family	NM_002866
0.18	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	M97287
0.19	cDNA Synthesis Control	
0.20	non-functional folate binding protein	NM_013307
6.37	perilipin	NM_002666
7.87	immunoglobulin lambda joining 3	NM_016934

Conclusion:

The UWB EMR exposure appears to affect the 244B cells at gene level compared to sham treated cells. By normalizing the screens using three different methods and then making a list of the common genes, we are able to reduce the number of false positives and false negatives.

PROJECT TITLE:

Analytical Comparison of genomic data from the results of the UWB TEMF on RNA transcription in 244B cells assessed using microarray (genomics) discovery methodology

Lead Investigator: Cynthia Galindo, B.S. Martin Meltz, PhD
Associated Investigators: Bijaya Nayak, M. Meltz

244B cells exposed to UWB EMR at 200 pps were harvested at 24 hr post exposure. The genomic profile from sham exposed cells was compared to that of UWB exposed cells. [The results of the three (3) different normalization approaches appear in Appendix A]

Using three different normalization methods and two different user-defined cutoff criteria, lists of genes were generated which met the defined criteria. The three different normalization methods include: 1.) No normalization 2.) User Defined normalization using selected Actin and GAPDH housekeeping genes and 3.) Global (default) normalization method using the intensity of all the spots on the array.

First, the Ratio intensity was set to 3.0 fold and difference values of absolute values was set to 0. For the no normalization method, the list includes 40 genes. The User defined normalization method yielded a list of 31 genes. The global (default) normalization method includes yielded a list of 28 genes. There are 22 genes in common between all three of the analysis reports. The list of genes and whether or not the gene is up-regulated or down-regulated compared to sham exposed controls is listed below. No absolute values are listed because the absolute numerical values differed depending on the normalization method.

Gene	Up- or Down-regulated
1. major histocompatibility complex, class I, A	Up-regulated
2. RAB3A, member RAS oncogene family	Up-regulated
3. ribosomal protein L19	Up-regulated
4. special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	Down-regulated
5. ribosomal protein, large, P0	Down-regulated
6. arylsulfatase A	Up-regulated
7. alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	Up-regulated
8. ribosomal protein L13a	Up-regulated
9. prothymosin, alpha (gene sequence 28)	Down-regulated
10. MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	Down-regulated
11. non-functional folate binding protein	Down-regulated
12. AKAP-binding sperm protein ropporin	Down-regulated
13. protein tyrosine phosphatase, non-receptor type 9	Up-regulated
14. perilipin	Up-regulated
15. KIAA0907 protein	Up-regulated
16. pleiotropic regulator 1 (PRL1homolog, Arabidopsis)	Up-regulated
17. interleukin 2 receptor, gamma (severe combined immunodeficiency)	Up-regulated
18. apoptosis inhibitor 5	Up-regulated
19. cytosolic ovarian carcinoma antigen 1	Up-regulated
20. immunoglobulin lambda joining 3	Up-regulated
21. ribosomal protein S9	Up-regulated
22. caspase 9, apoptosis-related cysteine protease	Down-regulated

The ratio intensity was changed to 5.0 fold and difference values of absolute values remained set to 0. For the no normalization method, the list includes 10 genes. The User defined normalization method yielded a list of 5 genes. The global (default) normalization method includes yielded a list of 6 genes. There are 3 genes in common between all three of the analysis reports. The list of genes and whether or not the gene is up-regulated or down-regulated compared to controls is listed below. No absolute values are listed because the absolute numerical values differed depending on the normalization method.

Gene	Up- or Down-regulated
1. RAB3A, member RAS oncogene family	Up-regulated
2. perilipin	Up-regulated
3. immunoglobulin lambda joining 3	Up-regulated

PROJECT TITLE:

Determination of p53 protein stabilization, loss of mitochondrial membrane potential, and release of cytochrome C into the cytosol in response to UWB EMR exposure in human lymphoblastoid cells.

Lead Investigator: Bijaya K Nayak, PhD
Assistants: Cynthia Galindo, Satnam P Mathur

Objective:

The aim of the study was to determine the effect of ultrawideband electromagnetic radiation (UWB EMR) exposure on p53 protein stabilization, loss in mitochondrial membrane potential, and the release of cytochrome C into the cytoplasm, which are molecular alterations associated either with induction of apoptosis or inhibition of cell cycle progression when DNA is damaged.

Methods:

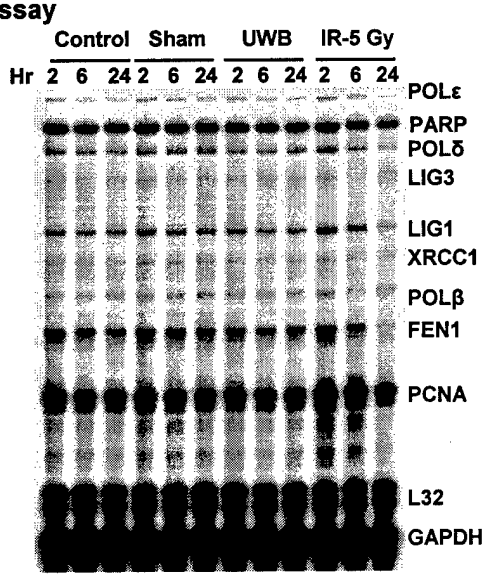
The studies were performed in 244B human lymphoblastoid cells and HL60cells. The cells were exposed to UWB EMR pulses intermittently for a total of 90 minutes (30 min on, 30 min off). The UWB EMR pulses had an average peak amplitude of 100 kV/m, an average pulse width of 0.80 ns, an average rise time of 200 ps, and a pulse repetition frequency of 250 pps. The frequencies ranged from D.C. to ~2 GHz. The stabilization of p53 protein was examined by western blot analysis. The transactivation of p53 target genes (p21, gadd45, Bax) was analyzed using the RNase protection assay. Further, evidence of the induction of apoptosis in response to UWB EMR exposure was determined by measuring a change in mitochondrial membrane potential using JC1 staining, and by detecting the release of cytochrome C into the cytoplasm (western blot analysis).

Results:

The p53 protein was not stabilized after the UWB EMR exposure of the 244B cells, i.e., there was no increase in the p53 protein level as compared to the sham and incubator control. There was no evidence of transcriptional induction of the p53 responsive genes p21, gadd45, and Bax after the UWB EMR exposure. In the positive control cells exposed to ionizing radiation, the p53 protein level was increased and there was an induction of the p53 target genes p21 and Bax. These are molecular responses associated with cell cycle arrest and apoptosis after this type of irradiation.
There was no loss of mitochondrial membrane potential and there was no release of cytochrome C into the cytoplasm in response to UWB EMR exposures (suggesting that apoptosis was not occurring), while in the positive control cells treated with staurosporine (1 μg/ml), there was a loss of mitochondrial membrane potential accompanied with the release of cytochrome C into the cytosol, indicating the onset of apoptosis.

Transcription of DNA Repair Genes

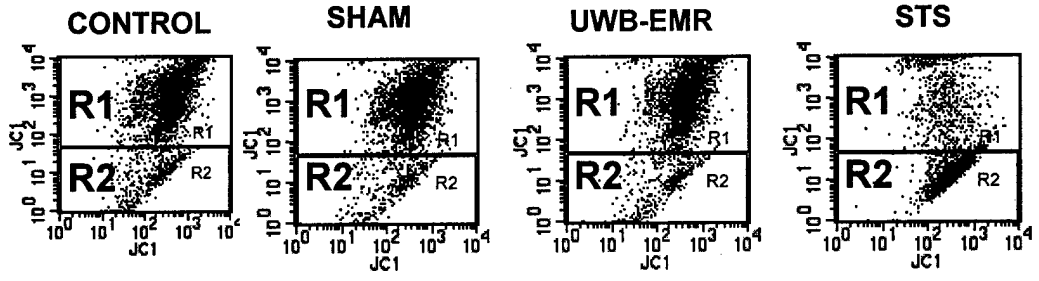
Cells: 244B
Exposure: HP UWB EMR
RNase Protection Assay



Determination of Mitochondrial Membrane Potential

Cells: 244B
Exposure: HP UWB EMR
Assay: JC1 Staining

R1: Live cells
R2: Dead cells

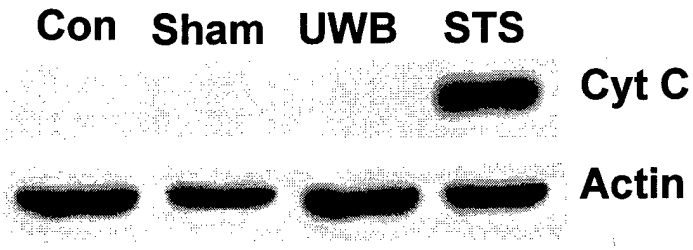


CONTROL	SHAM	UWB-EMR	STS
RI: 86%	RI: 87%	RI: 86%	RI: 28%
R2: 14%	R2: 13%	R2: 14%	R2: 72%

Release of Cytochrome C into the Cytoplasm

Cells: 244B
Exposure: HP UWB EMR
Assay: 10 hrs post-exposure

Western Blot



Conclusion:

The analysis of several different molecular parameters, such as p53 protein stabilization, loss of mitochondrial membrane potential, and cytochrome C release into the cytosol, indicates that after this type of UWB EMR exposure, induction of apoptosis and effects on cell cycle progression in human lymphoblastoid cells do not occur. The evidence is not supportive of the hypothesis that this type of exposure induces DNA strand breaks.

PROJECT TITLE:

NF-*kB* DNA-binding activity after High Peak Power Pulsed Microwave (8.2 GHz) Exposure of Normal Human Monocytes
Lead Investigator: Mohan Natarajan, Ph.D.
Associated Investigators: BK Nayak, AL Pandeswara, FN Roldan, C Galindo, ML Meltz, and S Mathur
This work has been published in Bioelectromagnetics 23:271 – 277 (2002).

PROJECT TITLE:

Nuclear translocation and DNA-binding Activity of NF-*kB* upon ultra-wideband electromagnetic radiation exposure fails to transactivate *kB*-dependent gene expression in human monocytes
Lead Investigator: Mohan Natarajan, Ph.D.
Associated Investigators: B.K. Nayak, S.L. Pandeswara, F.N. Roldan, C. Galindo, M.L. Meltz, S.P. Mathur

This work has been submitted to Radiation Research (2004).
See Attached Manuscript with the File Name:

> **Draft Manus Nuclear Transloc of NF-kB in MM6 cells after UWB EMR M Natarajan Aug 31, 2004**

PROJECT TITLE:

Dosimetry of UWB exposures of mammalian cells in tissue culture flasks positioned vertically, and perpendicular to the direction of propagation of the signal in a GTEMS cell.
Lead Investigator at UTHSCSA: Martin L. Meltz
Associated Investigator: Satnam Mathur
Lead Investigator at Univ. of Wisconsin-Madison: Susan C. Hagness, Ph.D. (Via subcontract), Department of Electrical and Computer Engineering
Associated Investigators: Zhen Ji (Post-doctoral Fellow) and John Booske

The first question raised, on behalf of the overall research project, was: **What is the exposure pattern for cells which are allowed to settle to the bottom of a partially filled flask?**

The second question raised, because of our desire to expose cells which grow surface attached was: **What is the exposure pattern for cells which are exposed surface attached, with the T-25 flask filled with medium so that the cells will be kept wetted during the UWB exposure in the GTEMS cell?**

Also of importance was the additional question: **Do the plastic of the walls of the T-25 flask, which were not modeled in the initial simulation, impact on the original analysis?**

The result was that they do not.

The Final Report for this project is presented in the attached power point presentation, with notes attached.
The file name is: gtem UWB Dosimetry Final Report Aug 30, 2004 Hagness.ppt

PROJECT TITLE:

RF Dosimetry and Temperature Distribution Analysis for performing 2.8 GHz High Peak Power Narrowband RF exposures

Project Leader at UTHSCSA: Martin L. Meltz
Project Leader at Brooks City-Base: Stewart Allen

The result of this study is that it will now be possible to expose T-25 flasks, containing either cells grown in suspension which have been allowed to settle, or cells grown surface attached, to pulsed high peak power 2.8 GHz narrowband RF, where the SARS will be known for each of the exposures to be performed.

The Final Report for this project is presented in the attached power point presentation.

The file name is:
2.8 GHz Narrowband Dosimetry Data Final Report Aug 30, 2004 S. Allen.ppt

APPENDIX A

THREE DIFFERENT NORMALIZATION METHODS APPLIED TO 244 B UWB EMR GENOMICS STUDY

072904 UWB EMR Exposure, 24hr post exposure

No Normalization, Ratio=3 , Difference=0 Analysis Report 1

Gene code	Intensity_1	Background_1	Adj.Intensity_1	Intensity_2	Background_2	Adj.Intensity_2	Ratio	Name	Protein/gene
A05ab6	190	27	163	1076	28	1048	6.43	major histocompatibility complex, class I, A	NM_002116
A07cd2	103	27	76	302	28	274	3.61	small nuclear ribonucleoprotein polypeptide E	NM_003094
A17gh3	97	27	70	278	28	250	3.57	hypothetical protein FLJ20054	NM_019049
A22gh1	199	133	66	259	28	231	3.50	WAS protein family, member 2	NM_006990
B04ef3	83	27	56	196	28	168	3.00	CGI-51 protein	NM_015380
B19cd4	130	27	103	349	28	321	3.12	UDP glycosyltransferase 2 family, polypeptide B15	NM_001076
B24ab8	97	27	70	895	28	867	12.39	RAB3A, member RAS oncogene family	NM_002866
C01ab2	124	66	58	247	28	219	3.78	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoa	NM_001607
C12ab6	1097	27	1070	4043	28	4015	3.75	ferritin, heavy polypeptide 1	NM_002032
C24ab2	107	27	80	332	28	304	3.80	arachidonate 15-lipoxygenase	NM_001140
D11gh2	137	27	110	369	28	341	3.10	hypothetical protein FLJ20608	NM_017900
D15cd1	378	27	351	2009	28	1981	5.64	ribosomal protein L19	NM_000981
E01ab2	142	27	115	378	28	350	3.04	aconitase 2, mitochondrial	NM_001098
E02ab8	393	27	366	1128	28	1100	3.01	purinergic receptor P2Y, G-protein coupled, 11	NM_002566
E07ef2	88	27	61	244	28	216	3.54	mitochondrial ribosomal protein L11	NM_016050
F01ab7	118	48	70	255	28	227	3.24	lamin A/C	NM_005572
F18ef6	408	27	381	121	28	93	0.24	special AT-rich sequence binding protein 1 (binds to nucle	M97287
F21cd1	2231	27	2204	746	28	718	0.33	ribosomal protein, large, P0	NM_001002
F24ab2	82	27	55	388	28	360	6.55	arylsulfatase A	NM_000487
G24ab2	87	27	60	309	28	281	4.68	alanyl (membrane) aminopeptidase (aminopeptidase N, an	NM_001150
H12ef1	1201	27	1174	6613	28	6585	5.61	ribosomal protein L13a	X56932
H12ef7	1053	27	1026	295	28	267	0.26	cDNA Synthesis Control	
H13ef5	331	27	304	126	28	98	0.32	prothymosin, alpha (gene sequence 28)	M26708
J21ab7	216	27	189	89	28	61	0.32	MCM6 minichromosome maintenance deficient 6 (MIS5 ho	NM_005915
J21ef4	1468	27	1441	408	28	380	0.26	non-functional folate binding protein	NM_013307
L08gh6	254	27	227	90	28	62	0.27	AKAP-binding sperm protein ropporin	NM_017578
N20ab8	97	27	70	273	28	245	3.50	RAD9 homolog (S. pombe)	NM_004584
N20ef1	277	27	250	840	28	812	3.25	cell death-regulatory protein GRIM19	NM_015965
N23ab8	100	27	73	462	28	434	5.95	protein tyrosine phosphatase, non-receptor type 9	NM_002833
O07ab6	339	27	312	1198	28	1170	3.75	eukaryotic translation elongation factor 1 alpha 1	NM_001402
O12ab8	133	27	106	935	28	907	8.56	perilipin	NM_002666
O12ef8	142	27	115	623	28	595	5.17	KIAA0907 protein	NM_014949
O18ab8	84	27	57	333	28	305	5.35	pleiotropic regulator 1 (PRL1homolog, Arabidopsis)	NM_002669
O23ef7	89	27	62	294	28	266	4.29	interleukin 2 receptor, gamma (severe combined immunod	D11086
P01ab2	144	27	117	525	28	497	4.25	apoptosis inhibitor 5	NM_006595
P03ab2	92	27	65	306	28	278	4.28	cytosolic ovarian carcinoma antigen 1	NM_006375
P08ef3	95	27	68	747	28	719	10.57	immunoglobulin lambda joining 3	NM_016934
P12cd5	881	27	854	4012	28	3984	4.67	ribosomal protein S9	U14971
P16ab3	295	27	268	110	28	82	0.31	caspase 9, apoptosis-related cysteine protease	NM_001229
P18gh7	146	27	119	388	28	360	3.03	hypothetical protein MGC3133	NM_031287
P23ab2	104	27	77	263	28	235	3.05	ADP-ribosylation factor 4-like	NM_001661

072904 UWB EMR Exposure, 24hr post exposure
User Defined Normalization (3 actin and 3 gapdh genes) Ratio=3 , Difference=0 Analysis Report 2

Gene code	Intensity_1	Background_1	Adj.Intensity_1	Intensity_2	Background_2	Adj.Intensity_2	Ratio	Name	Protein/gene
A05ab6	190	27	163	1076	28	884	5.42	major histocompatibility complex, class I, A	NM_002116
A06ab7	441	27	414	185	28	132	0.32	hepatocyte nuclear factor 3, gamma	NM_004497
A07cd2	103	27	76	302	28	231	3.04	small nuclear ribonucleoprotein polypeptide E	NM_003094
A17gh3	97	27	70	278	28	210	3.00	hypothetical protein FLJ20054	NM_019049
B24ab8	97	27	70	895	28	731	10.44	RAB3A, member RAS oncogene family	NM_002866
C01ab2	124	66	58	247	28	184	3.17	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoa	NM_001607
C12ab6	1097	27	1070	4043	28	3388	3.17	ferritin, heavy polypeptide 1	NM_002032
C24ab2	107	27	80	332	28	256	3.20	arachidonate 15-lipoxygenase	NM_001140
D15cd1	378	27	351	2009	28	1671	4.76	ribosomal protein L19	NM_000981
D21cd1	680	27	653	253	28	189	0.29	ribosomal protein L24	NM_000986
F18ef6	408	27	381	121	28	78	0.20	special AT-rich sequence binding protein 1 (binds to nucle	M97287
F21cd1	2231	27	2204	746	28	605	0.27	ribosomal protein, large, P0	NM_001002
F24ab2	82	27	55	388	28	303	5.51	arylsulfatase A	NM_000487
G24ab2	87	27	60	309	28	237	3.95	alanyl (membrane) aminopeptidase (aminopeptidase N, ar	NM_001150
H12ef1	1201	27	1174	6613	28	5557	4.73	ribosomal protein L13a	X56932
H12ef7	1053	27	1026	295	28	225	0.22	cDNA Synthesis Control	
H13ef5	331	27	304	126	28	82	0.27	prothymosin, alpha (gene sequence 28)	M26708
J13cd1	1702	27	1675	660	28	533	0.32	ribosomal protein S25	NM_001028
J21ab7	216	27	189	89	28	51	0.27	MCM6 minichromosome maintenance deficient 6 (MIS5 ho	NM_005915
J21ef4	1468	27	1441	408	28	320	0.22	non-functional folate binding protein	NM_013307
L08gh6	254	27	227	90	28	52	0.23	AKAP-binding sperm protein ropporin	NM_017578
N23ab8	100	27	73	462	28	366	5.01	protein tyrosine phosphatase, non-receptor type 9	NM_002833
O07ab6	339	27	312	1198	28	987	3.16	eukaryotic translation elongation factor 1 alpha 1	NM_001402
O12ab8	133	27	106	935	28	765	7.22	perilipin	NM_002666
O12ef8	142	27	115	623	28	502	4.37	KIAA0907 protein	NM_014949
O18ab8	84	27	57	333	28	257	4.51	pleiotropic regulator 1 (PRL1homolog, Arabidopsis)	NM_002669
O23ef7	89	27	62	294	28	224	3.61	interleukin 2 receptor, gamma (severe combined immunod	D11086
P01ab2	144	27	117	525	28	419	3.58	apoptosis inhibitor 5	NM_006595
P03ab2	92	27	65	306	28	234	3.60	cytosolic ovarian carcinoma antigen 1	NM_006375
P08ef3	95	27	68	747	28	606	8.91	immunoglobulin lambda joining 3	NM_016934
P12cd5	881	27	854	4012	28	3362	3.94	ribosomal protein S9	U14971
P16ab3	295	27	268	110	28	69	0.26	caspase 9, apoptosis-related cysteine protease	NM_001229

072904 UWB EMR Exposure, 24hr post exposure
Global (Sum Method) Normalization Ratio=1 , Difference=0 Analysis Report 3

Gene code	Intensity_1	Background_1	Adj.Intensity_1	Intensity_2	Background_2	Adj.Intensity_2	Ratio	Name	Protein/gene
A05ab6	190	27	163	1076	28	779	4.78	major histocompatibility complex, class I, A	NM_002116
A06ab7	441	27	414	185	28	116	0.28	hepatocyte nuclear factor 3, gamma	NM_004497
B24ab8	97	27	70	895	28	644	9.20	RAB3A, member RAS oncogene family	NM_002866
D15cd1	378	27	351	2009	28	1473	4.20	ribosomal protein L19	NM_000981
D21cd1	680	27	653	253	28	167	0.26	ribosomal protein L24	NM_000986
D24ef5	177	27	150	95	28	49	0.33	transferrin receptor (p90, CD71)	X01060
F18ef6	408	27	381	121	28	69	0.18	special AT-rich sequence binding protein 1 (binds to nucle	M97287
F21cd1	2231	27	2204	746	28	533	0.24	ribosomal protein, large, P0	NM_001002
F24ab2	82	27	55	388	28	267	4.85	arylsulfatase A	NM_000487
G24ab2	87	27	60	309	28	208	3.47	alanyl (membrane) aminopeptidase (aminopeptidase N, ar	NM_001150
H12ef1	1201	27	1174	6613	28	4897	4.17	ribosomal protein L13a	X56932
H12ef7	1053	27	1026	295	28	198	0.19	cDNA Synthesis Control	
H13ef5	331	27	304	126	28	72	0.24	prothymosin, alpha (gene sequence 28)	M26708
J09cd1	1787	27	1760	788	28	565	0.32	ribosomal protein S23	NM_001025
J13cd1	1702	27	1675	660	28	470	0.28	ribosomal protein S25	NM_001028
J15ef8	206	27	179	106	28	58	0.32	DKFZP564B167 protein	NM_015415
J21ab7	216	27	189	89	28	45	0.24	MCM6 minichromosome maintenance deficient 6 (MIS5 ho	NM_005915
J21ef4	1468	27	1441	408	28	282	0.20	non-functional folate binding protein	NM_013307
L08gh6	254	27	227	90	28	46	0.20	AKAP-binding sperm protein ropporin	NM_017578
N23ab8	100	27	73	462	28	322	4.41	protein tyrosine phosphatase, non-receptor type 9	NM_002833
O12ab8	133	27	106	935	28	674	6.36	perilipin	NM_002666
O12ef8	142	27	115	623	28	442	3.84	KIAA0907 protein	NM_014949
O18ab8	84	27	57	333	28	226	3.96	pleiotropic regulator 1 (PRL1homolog, Arabidopsis)	NM_002669
O23ef7	89	27	62	294	28	197	3.18	interleukin 2 receptor, gamma (severe combined immunod	D11086
P01ab2	144	27	117	525	28	369	3.15	apoptosis inhibitor 5	NM_006595
P03ab2	92	27	65	306	28	206	3.17	cytosolic ovarian carcinoma antigen 1	NM_006375
P08ef3	95	27	68	747	28	534	7.85	immunoglobulin lambda joining 3	NM_016934
P12cd5	881	27	854	4012	28	2962	3.47	ribosomal protein S9	U14971
P16ab3	295	27	268	110	28	60	0.22	caspase 9, apoptosis-related cysteine protease	NM_001229